Abstract
HIV eradication is hindered by the existence of latent HIV reservoirs in memory CD4+ T cells. T follicular helper (Tfh) cells are a subset of memory CD4 T cells present in lymph nodes and blood which harbor high frequencies of HIV-infected cells and likely play a role in HIV persistence. The objective of the current study was to investigate the role of Tfh cells in the establishment of latency using a primary cell model of HIV latency and the dual reporter virus, HIV DuoFluo. DuoFluo contains fluorescent markers under control of distinct promoters which are used to identify HIV LTR-driven transcription (GFP+; mKO2+/-; productive) and EF1α-driven transcription (mKO2+, GFP-; latent). For this study, tonsil tissue from HIV-negative donors was obtained as a source of lymphoid CD4+ T cells for in vitro infection experiments. Sorting of uninfected, latent, and productive-infected cells based on GFP and mKO2 expression was performed and cells were analyzed for surface expression of CD4, CXCR5, and PD1 and gene expression of a panel of 96 genes involved in T cell function and metabolism by Fluidigm Biomark assay. Tfh cells were found to be more permissive to latent infection compared to Non-Tfh cells. Targeted gene expression analysis revealed many transcriptional differences between productive and latent infection, but few between latent and uninfected cells. These studies will help elucidate mechanisms of latency establishment and will be valuable for design of better HIV/AIDS cure strategies.

Methods
Tonsil samples (n=8) were obtained from HIV-negative children and adolescents during elective tonsillectomy for sleep apnea at University of Miami Hospitals. Mononuclear cells were isolated from tissue and cryopreserved. Figure 3 shows the scheme for cell processing and infection with DuoFluo virus. Cells were sorted and analyzed using Sony SH800 Cell sorter. Cells were sorted directly into PCR buffer (CellsDirect, Life Tech) containing primers and enzymes for One-step cDNA synthesis and pre-amplification of target genes (Taqman, Life Tech). cDNA from sorted cell subsets were loaded in Fluidigm 96.96 gene expression IFC cartridge and standard microfluidic RT-PCR was performed.

Results
Gene expression of 96 targets in Tfh (PD1hiCXCR5hi) cells were assayed by intracellular fluorescence rather than virion production (Figure 7). This research was made possible by support from the Institute for AIDS and Emerging Infectious Diseases and the Miami CFAR at the University of Miami Miller School of Medicine funded by a grant (P30AI073961) from the NIH.

Conclusions
- Latency establishment occurs reproducibly at higher frequencies in lymphoid Tfh cells compared to Non-Tfh using in vitro primary cell model of latency.
- In this model, latent-infected Tfh cells are transcriptionally and phenotypically similar to uninfected Tfh cells, while productive-infected cells are transcriptionally distinct from both latent and uninfected cells.
- IL21, Tfh signature cytokine, gene expression is downregulated in both productive and latent infected Tfh cells which may have implications on overall function in HIV-infected Tfh cells.

References

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